

CLAIMS

WHAT IS CLAIMED IS:

1. A method for identifying inhibitors of a chlorophyll synthase (CS) enzyme, comprising:
 - a) incubating a CS polypeptide with a chlorophyllide and a phospholipid substrate in the presence and absence of a test compound under conditions suitable for CS activity;
 - b) adding to the incubation reactions a solution comprising a water immiscible organic solvent, a water-soluble alcohol, and a water-soluble dye that absorbs in the range of one or both of the excitation and emission wavelengths of the chlorophyllide substrate; and
 - c) measuring the fluorescence of the incubation reactions at from about 650 to 750nm, using from about 425 to 445nm as excitation wavelength, wherein a decrease in the fluorescence in the presence of the test compound indicates that the compound is a CS inhibitor.
2. The method of claim 1, wherein the CS is a plant CS.
3. The method of claim 2, wherein the plant is a dicot.
4. The method of claim 2, wherein the plant is a monocot.
5. The method of claim 2, wherein the CS is an *Arabidopsis* CS.
6. The method of claim 2, wherein the CS is SEQ ID NO:1.
7. The method of claim 2, wherein the CS is a CS polypeptide consisting essentially of SEQ ID NO:1.
8. The method of claim 1, wherein the CS is a fungal CS.

9. The method of Claim 1 wherein the fluorescence is measured at about 665nm using about 440nm as excitation wavelength.
10. The method of Claim 1 wherein the water immiscible organic solvent is dodecane, the water-soluble dye is Malachite Green, the water-soluble alcohol is ethanol and the phospholipid substrate is geranylgeranyl diphosphate.
11. A method for identifying inhibitors of a chlorophyll synthase (CS) enzyme, comprising:
 - a) incubating a CS polypeptide with a chlorophyllide and a phospholipid substrate in the presence of a water-soluble dye that absorbs in the range of one or both of the excitation and emission wavelength ranges of the chlorophyllide substrate, and in the presence and absence of a test compound under conditions suitable for CS activity;
 - b) adding to the incubation reactions a solution comprising a water immiscible organic solvent and a water-soluble alcohol; and
 - c) measuring the fluorescence of the incubation reactions at from about 650 to 750nm, using from about 425 to 445nm as excitation wavelength, wherein a decrease in the fluorescence in the presence of the test compound indicates that the compound is a CS inhibitor.
12. The method of claim 11, wherein the CS is a plant CS.
13. The method of claim 12, wherein the plant is a dicot.
14. The method of claim 12, wherein the plant is a monocot.
15. The method of claim 12, wherein the CS is an *Arabidopsis* CS.
16. The method of claim 12, wherein the CS is SEQ ID NO:1.

17. The method of claim 12, wherein the CS is a CS polypeptide consisting essentially of SEQ ID NO:1.
18. The method of claim 11, wherein the CS is a fungal CS.
19. The method of Claim 11 wherein the fluorescence is measured at about 665nm using about 440nm as excitation wavelength.
20. The method of Claim 11 wherein the water immiscible organic solvent is dodecane, the water-soluble dye is Malachite Green, the water-soluble alcohol is ethanol and the phospholipid substrate is geranylgeranyl diphosphate.
21. A method for concurrently testing a plurality of compounds as inhibitors of a chlorophyll synthase (CS) enzyme, comprising:
 - a) incubating a plurality of test compounds in a multi-well format, individually or in mixtures, with a CS polypeptide and a chlorophyllide and phospholipid substrate under conditions suitable for CS activity;
 - b) incubating in at least one of the wells the CS polypeptide and the substrates under conditions suitable for CS activity in the absence of a test compound;
 - c) adding to the incubation reactions of each of the wells a solution comprising a water immiscible organic solvent, a water-soluble alcohol and a water-soluble dye that absorbs in the range of one or both of the excitation and emission wavelength ranges of the chlorophyllide substrate;
 - d) measuring the fluorescence of the wells from about 650 to 750nm using from about 425 to 445nm as excitation wavelength; and
 - e) comparing the fluorescence of the wells comprising the CS in the presence and in the absence of the test compound(s), wherein a relative decrease in fluorescence for the wells comprising the test compound(s), indicates that at least one of the test compounds comprised within is a CS inhibitor.

22. The method of claim 21, wherein the CS is a plant CS.
23. The method of claim 22, wherein the plant is a dicot.
24. The method of claim 22, wherein the plant is a monocot.
25. The method of claim 22, wherein the CS is an *Arabidopsis* CS.
26. The method of claim 22, wherein the CS is SEQ ID NO:1.
27. The method of claim 22, wherein the CS is a CS polypeptide consisting essentially of SEQ ID NO:1.
28. The method of claim 21, wherein the CS is a fungal CS.
29. The method of Claim 21 wherein the fluorescence is measured at about 665nm using about 440nm as excitation wavelength.
30. The method of Claim 21 wherein the water immiscible organic solvent is dodecane, the water-soluble dye is Malachite Green, the water-soluble alcohol is ethanol and the phospholipid substrate is geranylgeranyl diphosphate.
31. A method for concurrently testing a plurality of compounds as inhibitors of a chlorophyll synthase (CS) enzyme, comprising:
- a) incubating a plurality of test compounds in a multi-well format, individually or in mixtures, with a CS polypeptide and a chlorophyllide and phospholipid substrate under conditions suitable for CS activity, and with a water-soluble dye that absorbs in the range of one or both of the excitation and emission wavelength ranges of the chlorophyllide substrate;

- b) incubating in at least one of the wells the CS polypeptide, the substrates and the water-soluble dye under conditions suitable for CS activity in the absence of a test compound;
- c) adding to the incubation reactions of each of the wells a solution comprising a water immiscible organic solvent and a water-soluble alcohol;
- d) measuring the fluorescence of the wells from about 650 to 750nm using from about 425 to 445nm as excitation wavelength; and
- e) comparing the fluorescence of the wells comprising the CS in the presence and in the absence of the test compound(s), wherein a relative decrease in fluorescence for the wells comprising the test compound(s), indicates that at least one of the test compounds comprised within is a CS inhibitor.

32. The method of claim 31, wherein the CS is a plant CS.

33. The method of claim 32, wherein the plant is a dicot.

34. The method of claim 32, wherein the plant is a monocot.

35. The method of claim 32, wherein the CS is an *Arabidopsis* CS.

36. The method of claim 32, wherein the CS is SEQ ID NO:1.

37. The method of claim 32, wherein the CS is a CS polypeptide consisting essentially of SEQ ID NO:1.

38. The method of claim 31, wherein the CS is a fungal CS.

39. The method of Claim 31 wherein the fluorescence is measured at about 665nm using about 440nm as excitation wavelength.

40. The method of Claim 31 wherein the water immiscible organic solvent is dodecane, the water-soluble dye is Malachite Green, the water-soluble alcohol is ethanol and the phospholipid substrate is geranylgeranyl diphosphate.